Transmission of Venezuelan Equine Encephalomyelitis Virus by Strains of *Aedes albopictus* (Diptera: Culicidae) Collected in North and South America

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ABSTRACT Experimental studies were undertaken to ascertain the vector potential of North American (Houston and Alsace) and South American (Sao Paulo and Santa Teresa) strains of *Aedes albopictus* (Skuse) for an epizootic (Trinidad donkey) strain of Venezuelan equine encephalomyelitis (VEE) virus. Infection rates were similar in all four strains of *Ae. albopictus* tested after ingestion of VEE virus from a viremic hamster. Virus disseminated from the midgut to the hemocoel in about 80% of infected mosquitoes, regardless of the dose ingested ($10^{4.6}$ to $10^{5.7}$ plaque-forming units per mosquito) or the time of extrinsic incubation (7–35 d). Although all four strains of this mosquito transmitted VEE virus by bite to hamsters, transmission rates were significantly higher for the South American strains (24%, 40 of 170) than for the North American strains (5%, 9 of 165). Although VEE virus has never been isolated from *Ae. albopictus*, the introduction of this species into the Americas may allow it to serve as an amplification vector in areas where epizootic strains of VEE are found or introduced.

KEY WORDS Insecta, *Aedes albopictus*, Venezuelan equine encephalomyelitis virus, vector potentials

THE RECENT INTRODUCTION of *Aedes albopictus* (Skuse) into the Americas has raised a concern that these mosquitoes may serve as a vector for endemic as well as exotic viruses (Knudson 1986). Laboratory studies have demonstrated the ability of this species to transmit numerous arboviruses, including some native to the Americas (Shroyer 1986, Hawley 1988). In addition, *Ae. albopictus* can displace populations of *Aedes aegypti* (L.) in urban environments (Black et al. 1989) and has demonstrated an ability to flourish in arboreal settings (Rai 1986).

North and South American strains of *Ae. albopictus* differ from each other in several life history characteristics, including egg diapause, photoperiod sensitivity, and bloodmeal size (G. B. Craig, personal communication). Differences in vector competence for dengue viruses between North and South American strains also have been reported (Miller & Ballinger 1988). The strains of *Ae. albopictus* introduced into North America are presumed to have originated in temperate Asia, possibly Japan (Hawley et al. 1987). In contrast, strains introduced into South America may have come from a tropical region of Asia (Hawley 1988).

Venezuelan equine encephalomyelitis (VEE) virus has caused sporadic epizootics of severe disease, usually fatal in horses and occasionally so in man, primarily in Central America (Walton & Grayson 1989). Epizootics have extended into northern South America and have occurred as far north as Texas in 1969–1972. Because of the potential for *Ae. albopictus* to transmit VEE virus, we evaluated two North American and two South American strains of *Ae. albopictus* for their ability to transmit an epizootic variant of VEE virus under laboratory conditions.

Materials and Methods

Mosquitoes. North American strains of *Ae. albopictus* were the Houston strain (Mitchell et al. 1987), derived from specimens collected in Houston, Tex., and obtained from Carl Mitchell, Centers for Disease Control, Ft. Collins, Colo.; and the Alsace strain, derived from specimens collected in Indiana and obtained from George Craig, Jr., University of Notre Dame, South Bend, Ind. (Table 1). South American strains were derived from specimens collected in Sao Paulo, Sao Paulo and Santa Teresa, Espirito Santo, Brazil (Sao Paulo and Santa Teresa, respectively). These strains also were obtained from Dr. Craig. Mosquitoes were maintained at 26°C using procedures described by Gargan et al. (1983), and

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Table 1. Strains of *Ae. albopictus* tested in infection and transmission experiments

Strain	Collection location (year collected)	Generation
Houston	Houston, Tex. (1985)	F_5–F_9
Alsace	New Alsace, Ind. (1987)	F_2–F_5
Santa Teresa	Santa Teresa, Espirito Santo, Brazil (1987)	F_2–F_5
Sao Paulo	Sao Paulo, Sao Paulo, Brazil (1987)	F_2–F_5

female mosquitoes were 4–10 d old when used for infection trials.

Virus and Virus Assay. A plaque-purified clone (FC-1-1) of the epizootic VEE subtype 1A Trinidad donkey strain (Johnston & Smith 1988) was used throughout these studies. It was passaged twice in BHK 21 cells and once in Vero cells after it had been cloned.

Serial 10-fold dilutions of specimens were plaque-assayed for infectious virus on Vero cell monolayers.

Determination of Vector Potential. Mosquitoes were allowed to feed on anesthetized female Syrian hamsters that had been inoculated intraperitoneally 96 h earlier with 0.2 ml of a suspension containing $10^{8.5}$ plaque-forming units (PFU) of VEE virus. In most feedings, the anesthetized hamster was placed so that both a North American and a South American strain of *Ae. albopictus* could feed on it concurrently, thus insuring equal virus exposure to both strains. Immediately after feeding, five engorged mosquitoes from each hamster were triturated individually in 1 ml of diluent (10% fetal bovine serum in Medium 199 with Hanks' salts and antibiotics), frozen at −70°C, and then assayed on Vero cell monolayers to determine the amount of virus ingested. The remaining engorged mosquitoes were placed in a 3.8-liter cardboard container with netting on one end. Apple slices or a 7% sucrose solution was provided as a carbohydrate source. An oviposition substrate was added 4 d after the infectious blood meal. At 7-d intervals after the infectious blood meal, transmission attempts were made by allowing a sample of mosquitoes to feed, either individually or in pools of up to five mosquitoes each, on susceptible hamsters. Immediately after each transmission trial, mosquitoes were cold-anesthetized, their legs and bodies triturated separately in 1 ml of diluent, and frozen at −70°C.

Infection was determined by the recovery of virus from the mosquito body tissue samples at ≥7 d after the infectious blood meal. A mosquito that had virus recovered from its body (but not its legs) was considered to have a nondisseminated infection limited to its midgut. In contrast, if virus was recovered from both body and leg suspensions, the mosquito was considered to have a disseminated infection (Turell et al. 1984). Because infection with VEE virus is virtually 100% fatal for hamsters, hamster death was used as the criterion for viral transmission. Isolation of virus from liver or brain tissue samples, or both, verified transmission. Any hamster that did not die after being fed upon by a mosquito with a disseminated infection was challenged with $10^{8.5}$ PFU of virus to confirm its susceptibility.

Table 2. Infection and dissemination rates in selected strains of *Ae. albopictus* after ingestion of $10^{4.6}$–$10^{5.7}$ PFU of Venezuelan equine encephalomyelitis virus

Strain	No. tested	No. infected (%)	No. disseminated (%)
Houston	127	68 (54)	50 (39)
Alsace	91	67 (74)	53 (58)
Total, U.S. strains	218	135 (62)	103 (47)
Santa Teresa	189	133 (70)	110 (58)
Sao Paulo	168	104 (62)	84 (50)
Total, Brazilian strains	357	237 (66)	194 (54)

Results

Mosquitoes ingested $10^{4.6}$–$10^{5.7}$ PFU of VEE virus in the various feedings. Within each strain, no consistent differences were observed in infection, dissemination, or transmission rates, regardless of the dose of VEE virus ingested. Thus, data for each strain were combined for further analysis. Infection and dissemination rates were similar in all of the Brazilian and North American strains tested (Table 2), but transmission rates differed (Tables 3 and 4). Transmission was first observed on day 7 after the infectious blood meal, and transmission rates were consistently higher at each time period for the Brazilian strains compared with the U.S. strains (Table 3). Transmission rates by Brazilian strains (24%, 40 of 170), were significantly higher ($\chi^2 = 20.5$; df = 1; $P < 0.001$) than by U.S. strains (5%, 9 of 165), and this difference could be attributed to the percentage of mosquitoes with a disseminated infection that transmitted virus (Table 4).

For both the North and South American strains, the virus titers recovered from mosquitoes with a disseminated infection were similar for transmitting ($10^{5.7}$ and $10^{5.6}$ PFU per mosquito, respectively) and nontransmitting ($10^{5.8}$ and $10^{5.7}$ PFU per mosquito, respectively) individuals. Also, the distribution of titers recovered from all individual infected mosquitoes was similar for the North and South American strains (Fig. 1).

Discussion

Strains of *Ae. albopictus* derived from specimens collected in both North and South America were competent vectors of VEE virus. Although both infection and dissemination rates, as well as the

Table 3. Transmission rates[a] by day of extrinsic incubation in selected strains of *Ae. albopictus* after ingestion of $10^{4.6}$–$10^{5.7}$ PFU of Venezuelan equine encephalomyelitis virus

Strain	Day of extrinsic incubation				Totals
	7	14	21	≥28	
Houston	1/39 (3%)	3/25 (12%)	0/11 (0%)	1/18 (6%)	5/93 (5%)
Alsace	0/41 (0%)	3/25 (12%)	1/6 (17%)	—	4/72 (6%)
Total, U.S. strains	1/80 (1%)	6/50 (12%)	1/17 (6%)	1/18 (6%)	9/165 (5%)
Santa Teresa	3/22 (14%)	6/27 (22%)	5/11 (45%)	5/18 (27%)	19/78 (24%)
Sao Paulo	4/28 (14%)	6/25 (24%)	8/21 (38%)	3/18 (17%)	21/92 (23%)
Total, Brazilian strains	7/50 (14%)	12/52 (23%)	13/32 (41%)	8/36 (22%)	40/170 (24%)

[a] Number of mosquitoes transmitting/number refeeding (percentage transmitting).

distribution of VEE viral titers recovered from individual mosquitoes, were similar, the South American strains were significantly more efficient laboratory vectors of VEE virus than were the North American strains. This is in contrast to the greater susceptibility to all four serotypes of dengue viruses by the Houston strain of *Ae. albopictus* compared with a Brazilian strain of *Ae. albopictus* (Miller & Ballinger 1988).

The difference in transmission rates between the North and South American strains of *Ae. albopictus* in the present study probably is due to either a salivary gland infection or a salivary gland escape barrier (Kramer et al. 1981) because only 16% of the females of the U.S. strains with a disseminated infection transmitted VEE virus compared with 60% of the disseminated refeeding Brazilian mosquitoes. Interestingly, for mosquitoes with a disseminated infection, there were no significant differences in titers recovered from a mosquito, regardless of its transmission status (transmitter or nontransmitter) or its origin (North or South America).

The transmission rate we observed for the South American strains (24%, 40 of 170), is similar to that reported for *Psorophora confinnis* (Lynch Arribalzaga) (33%, 6 of 18) (Sudia et al. 1971b). This species has been implicated as a natural vector of epizootic VEE virus (Sudia et al. 1971a) and, of all the mosquito species tested, was the most efficient transmitter for an epizootic strain of VEE virus (Sudia et al. 1971b).

The hamster viremias to which mosquitoes were exposed in the present study are comparable with those observed in burros (Gochenour et al. 1962) and in horses (Kissling et al. 1956, Sudia et al. 1971b) inoculated with an epizootic strain of VEE virus. *Ae. albopictus* readily feeds on humans, as evidenced by its role in the transmission of dengue virus (Metselaar et al. 1980). In addition, it avidly feeds on many other mammals, including horses (Tempelis et al. 1970). This mosquito has extended its range in many areas, including areas where VEE has been epizootic in North America. Thus, *Ae. albopictus*, especially the Brazilian strains, should be considered a potential vector of epizootic VEE virus.

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Table 4. Transmission rates in selected strains of *Ae. albopictus* after ingestion of $10^{4.6}$–$10^{5.7}$ PFU of Venezuelan equine encephalomyelitis virus

Strain	All refeeding[a]	Disseminated, refeeding[b]
Houston	5/93 (5%)	5/26 (19%)
Alsace	4/72 (6%)	4/31 (13%)
Total, U.S. strains	9/165 (5%)	9/57 (16%)
Santa Teresa	19/78 (24%)	19/33 (58%)
Sao Paulo	21/92 (23%)	21/33 (64%)
Total, Brazilian strains	40/170 (24%)	40/66 (61%)

[a] Number of mosquitoes transmitting/number refeeding (percentage transmitting).

[b] Number of mosquitoes transmitting/number with a disseminated infection refeeding (percentage transmitting).

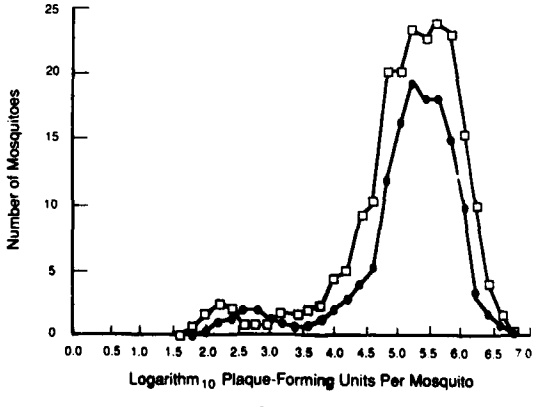

Fig. 1. Distribution of viral titers recovered from North and South American strains of *Ae. albopictus* tested 7–35 d after ingestion of VEE virus. □, South American strains. ●, North American strains.

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